500	10*	25*
Purified Water	1.5	3.5
Lomefloxacin		
49	2.5	26*
70	1	31*
100	1.5	29.5*
0.1 M NaOH	1	1.5
-8-MOP, 1	2	44.5*
NQO, 0.125	34.5*	---

*p≤0.001 (Fisher's Exact Test)
**p≤0.01 (Fisher's Exact Test)

SB-265805-S: A Study to Investigate Effects on Bone Marrow Following Oral Administration to Rats (SB Document No. SB-265805/RSD-1014PP/1; Protocol No. G99594)

E. Nicholas, R. Lambert, A. Pritchard, J. Fuller, A. Porter, R. McCarthy (SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Herts, UK)

Report dated: 10/21/99, UK, US, and OECD GLP

Vol. 1.5.028

Animals: Male Sprague-Dawley rats (Crl:CD(SD) IGS BR); 10 weeks old and 336-443g at the initiation of dosing; 7 per group for bone marrow investigation and satellite groups of 3 for toxicokinetics

Diet: SQC Rat and Mouse Maintenance Diet No. 1 [redacted] and filtered tap water were available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin (Batch No. EF03-14R1P5) was dissolved in 0.9% aqueous saline and administered via oral gavage for 2 consecutive days at 0 (vehicle control), 200, 400, 800, 1200, or 1600 mg/kg/day at a dose volume of 10 ml/kg.

Length and Conduct of Study: Rats were sacrificed 24 hours after the final dose of drug was given. Femoral bone marrow was harvested and smears were made on microscope slides. The slides were air dried, fixed in methanol and stained (Modified Wright's stain for myelograms and Acridine Orange, pH 7.4, for micronuclei). The slides were coded for blinded examination. For micronuclei slides, 2000 polychromatic erythrocytes (PCEs) from at least 2 slides were observed for each animal. The percentage of PCEs containing micronuclei was determined, as well as the ratio of PCEs

to total erythrocytes (indicative of bone marrow toxicity). A concurrent positive control was not run in this experiment, but positive control slides (cyclophosphamide) from another experiment were included in the staining and reading processes for quality control. For myelogram slides, 200 consecutive cells were counted and classified as follows: erythrocyte series, myeloid series, lymphocytes, monocytes, plasma cells, megakaryocytes, reticular cells, and megakaryoblasts. Those from the erythrocyte or myeloid series were subclassified according to stage of maturation and lineage. Only the negative control and high dose groups had their myelogram slides analyzed- the other groups were not done since no gemifloxacin-related effects were observed at the high dose.

Blood samples were drawn immediately prior to sacrifice of the rats assigned for bone marrow evaluation and general hematologic parameters were measured.

Blood samples for toxicokinetic analysis were drawn from the tail veins into EDTA immediately before the second gemifloxacin dose was given, and 0.5, 1, 2, 4, 8, 12, and 24 hours after the second dose was administered. Plasma was analyzed for gemifloxacin concentration using a validated ——— method with a lower limit of detection of ——— g/ml.

Results: No unscheduled deaths occurred. Reduced activity and/or piloerection were seen in 2 rats at 1200 mg/kg on day 2 of dosing. A slight loss of body weight was observed in the 1200 and 1600 mg/kg rats. There were some drug-related changes in general hematologic parameters (peripheral blood samples from rats assigned for bone marrow evaluation). At doses of gemifloxacin ≥800 mg/kg/day, there were decreases in the group mean reticulocyte counts (17, 30, and 32% at 800, 1200, and 1600 mg/kg, respectively) compared to controls. Average absolute counts of eosinophils and basophils were decreased by 47 and 43% in the 1600 mg/kg/day group. Group mean hemoglobin concentrations, hematocrits, and red cell counts increased slightly (about 5-7%) in all gemifloxacin-treated groups compared to controls, but the increases were not dose-related. The group mean total white blood cell counts in all gemifloxacin-treated groups were all less than control (10-25%), but the reduction was not dose-related. The reductions in group mean total lymphocyte counts (up to about 36% at the 2 highest dose groups) seen in the gemifloxacin-treated rats did tend to be dose related.

Gemifloxacin induced micronucleus formation in the bone marrow of the rats at doses of ≥800 mg/kg/day. There were dose-related increases in both bone marrow toxicity (reduction in the percentage of PCEs out of the total number of erythrocytes) and micronucleus formation (the percentage of PCEs that contained micronuclei).

Gemifloxacin-Induced Micronucleus Formation in Rat Bone Marrow (Average ± SD)

Dose (mg/kg/day)	% PCE with Micronuclei	%PCE (out of Total Erythros)
0 (Vehicle Control)	0.36 ± 0.15	32.10 ± 8.20
200	0.46 ± 0.09	29.81 ± 9.86

400	0.40 ± 0.13	28.86 ± 11.75
800	1.18 ± 0.46*	22.23 ± 4.74
1200	1.24 ± 0.53*	18.07 ± 3.86
1600	2.16 ± 1.09*	15.77 ± 8.77

*Significant increase in micronucleated PCE (>3-fold of control)

Toxicokinetic evaluation demonstrated that Cmax increased in a dose proportional manner up to 1200 mg/kg, but Cmax was not higher at 1600 mg/kg than it was at 1200 mg/kg. Gemifloxacin exposure (based upon AUC) increased in a roughly dose proportional manner up to 1600 mg/kg. At the no-effect dose for micronucleus formation (400 mg/kg), the Cmax for gemifloxacin in the rats was 5.47 g/ml and the AUC was 33.2 gh/ml.

Toxicokinetic Parameters for Gemifloxacin in Rats After 2 Daily Oral Doses (Average ± SD)

Dose (mg/kg/day)	Tmax (h)*	Cmax (g/ml)	$AUC_{0\text{-}24\,h}$ (gh/ml)
200	2.02 (2.02-8.00)	3.34 ± 0.62	28.3 ± 2.9
400	0.50 (0.50-0.50)	5.47 ± 0.82	33.2 ± 7.1
800	1.00 (0.50-1.00)	9.11 ± 2.31	85.7 ± 33.8
1200	1.02 (1.00-1.03)	17.5 ± 7.8	137 ± 39
1600	1.03 (1.03-2.05)	16.5 ± 4.2	238 ± 44

*Tmax is expressed as the median, with the range in parentheses

Gemifloxacin induced micronucleus formation in the bone marrow of rats following oral administration of the drug for 2 consecutive days at doses of ≥800 mg/kg/day. The no effect level for micronucleus formation was 400 mg/kg/day. The doses of gemifloxacin associated with micronucleus formation occurred were toxic to the bone marrow. The percentage of PCEs (out of the total number of erythrocytes) in the bone marrow was clearly reduced at ≥800 mg/kg/day, as was the reticulocyte count in peripheral blood. The report mentioned that reticulocyte count might be a useful surrogate for gemifloxacin-induced bone marrow toxicity in situations (i.e., a human clinical trial) where a less invasive method to monitor bone marrow toxicity would be desirable. The report authors are apparently assuming that micronucleus formation does not occur at doses of gemifloxacin that are not toxic to the bone marrow; thus if no evidence of bone marrow toxicity (reduction of reticulocyte count) is observed in humans, micronucleus formation would be unlikely to occur at doses of gemifloxacin used clinically. Human studies (both sexes) using repeated oral gemifloxacin daily doses of 320 mg/day have demonstrated an average Cmax of approximately 1.5 g/ml (range from 0.7-2.6 g/ml) and average AUC of about 9 gh/ml (range from 4.7-20.1 gh/ml). The report warns against using either Cmax or AUC in isolation to try to predict *in vivo* micronucleus formation. In a rat micronucleus study that used an intravenous route of administration (see report immediately below), the no-effect dose for gemifloxacin was

20 mg/kg which gave a Cmax of 31.1 ± 3.72 g/ml and an AUC of 13.8 ± 1.35 gh/ml. This Cmax is about 3 times higher than that observed for the lowest dose that induced micronuclei in the oral study (800 mg/kg), although the AUC after the oral 800 mg/kg dose was over twice as high as that observed following the 20 mg/kg IV dose. The lowest gemifloxacin dose in the IV rat study that induced micronuclei was 40 mg/kg, giving a Cmax of 63.6 ± 20.1 g/ml and an AUC of 35.2 ± 3.23 gh/ml. This AUC is comparable to that observed at the no effect dose in the oral rat micronucleus study (400 mg/kg), but the Cmax after the 40 mg/kg IV dose was over 5 times higher than that observed after the 400 mg/kg oral dose.

Intravenous Micronucleus Assay in Rats (SB Document No. SB-265805/RSD-10110J/1; Protocol No. G99529)

R. Lambert, E. Nicholas, A. Pritchard, J. Fuller, A. Porter, R. McCarthy (SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Herts, UK)

Report dated: 10/14/99, UK, US, and OECD GLP

Vol. 1.5.028

Animals: Male Sprague-Dawley rats (Crl:CD(SD) IGS BR); 8-9 weeks old and 261-392 g at the initiation of dosing; 7 per group for bone marrow investigation with satellite groups of 4 for concurrent toxicokinetics; 3 for positive control; 4 per group for second toxicokinetic study
Diet: SQC Rat and Mouse Maintenance Diet No. 1 [redacted] and filtered tap water were available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin (Batch No. EF03-14R1P5) was dissolved in 0.9% aqueous saline and administered intravenously to a lateral tail vein as a bolus for 2 consecutive days at 0 (vehicle control), 10, 20, 40, or 80 mg/kg/day at a dose volume of 5 ml/kg. A positive control group received a single oral dose of 75 mg/kg cyclophosphamide (vehicle, sterile water; dose volume, 10 ml/kg). The gemifloxacin doses were chosen based upon the results of a range finding study that used 6-100 mg/kg of drug. In this range finding study, bone marrow toxicity (reduction in the percentage of PCE out of total erythrocytes) was observed at ≥80 mg/kg, with marked bone marrow hypocellularity at 100 mg/kg.

Length and Conduct of Study: Rats were sacrificed 24 hours after the final dose of drug was given. Femoral (for micronuclei) and tibial (for M:E ratio) bone marrow was harvested and smears were made on microscope slides. The slides were air dried, fixed in methanol and stained (Modified Wright's stain for M:E ratio- performed under protocol no. G99582, but reported here) and Acridine Orange, pH 6.4, for micronuclei). The slides were coded for blinded examination. For micronuclei slides, 2000 polychromatic erythrocytes (PCEs) from at least 2 slides were observed for each animal.

The percentage of PCEs containing micronuclei was determined, as well as the ratio of PCEs to total erythrocytes (indicative of bone marrow toxicity). For M:E ratio, myeloid and erythroid cells were classified and the ratio calculated (total number of cells classified not stated).

Blood samples for toxicokinetic analysis performed concurrently with the bone marrow evaluation were drawn from a tail vein other than that used for dosing into EDTA 1, 10, and 30 minutes and 2, 4, 8, and 24 hours after the second dose was administered. Plasma was analyzed for gemifloxacin concentration using a validated ——method with a lower limit of detection of —— g/ml.

The investigators were concerned that Cmax was underestimated in the toxicokinetics portion of the micronucleus study, so another toxicokinetic study was conducted using the same doses of gemifloxacin (2 doses given 24 h apart). In this second study, rats were implanted with cannulas in their jugular and femoral veins. Gemifloxacin was given to the animals via the femoral vein cannula by controlled rapid intravenous infusion (5 ml/kg over 30 seconds). Blood samples were taken from the jugular vein cannula at the end of the second infusion (within 1 minute), and 2.5, 5, 10, 15 and 30 minutes and 2, 4, 8, and 24 hours after the second infusion was completed. EDTA was also used as an anticoagulant in this study and the gemifloxacin concentrations in the plasma were measured as above.

Results: Slight body weight loss was observed at doses ≥40 mg/kg. No unscheduled deaths occurred and no other clinical signs of toxicity were observed.

Gemifloxacin induced micronucleus formation in the bone marrow of the rats at doses of 40 and 80 mg/kg/day. Bone marrow toxicity manifested by a reduction in the percentage of PCEs out of the total number of erythrocytes was evident at both doses. At 80 mg/kg/day, a reduction in the M:E ratio was also observed and was attributed to a relative decrease in erythroid cells (based on reduced reticulocyte counts that were seen in other gemifloxacin studies). The positive and negative controls performed acceptably.

Gemifloxacin-Induced Micronucleus Formation in Rat Bone Marrow (Average ± SD)

Dose (mg/kg/day)	% PCE with Micronuclei	%PCE (out of Total Erythros)
0 (Vehicle Control)	0.22 ± 0.12	36.20 ± 13.28
10	0.30 ± 0.21	31.5 ± 5.04
20	0.38 ± 0.17	32.48 ± 10.27
40	1.09 ± 0.18*	27.27 ± 6.33
80	4.86 ± 0.49*	8.21 ± 3.86
Cyclophosphamide	1.78 ± 0.33	14.79 ± 7.18

*Statistically significant compared to vehicle control based upon a one-way Analysis of Variance and Dunnett's procedure. Positive control data were not subjected to formal statistical analysis.

The results of the toxicokinetics portion of the micronucleus study were highly variable, with neither Cmax nor AUC increasing in a dose-proportional manner. Thus, the investigators decided to perform a controlled infusion toxicokinetics study using the same doses of drug. The results from that study were much less variable, with roughly dose-proportional increases in Cmax and AUC.

Toxicokinetics of Gemifloxacin in Rats After 2 Daily IV Doses-Micronucleus Study (Average ± SD)

Dose (mg/kg/day)	Cmax (g/ml)	AUC (gh/ml)
10	110 ± 97.3	16.0 ± 9.90
20	27.6 ± 3.13	16.8 ± 0.829
40	229 ± 119	79.1 ± 39.1
80	63.0 ± 8.91	78.3 ± 4.40

Toxicokinetics of Gemifloxacin in Rats After 2 Daily IV Doses-Controlled Infusion Study (Average ± SD)

Dose (mg/kg/day)	Cmax (g/ml)	AUC (gh/ml)
10	13.3 ± 4.05	5.26 ± 1.68
20	31.1 ± 3.72	13.8 ± 1.35
40	63.6 ± 20.1	35.2 ± 3.23
80	159 ± 22.4	80.0 ± 6.15

Gemifloxacin induced micronucleus formation in rat bone marrow PCEs following 2 days of intravenous dosing at 40 and 80 mg/kg/day. Bone marrow toxicity (reduction of the % PCE out of total erythrocytes) was also observed at these doses. The no-effect gemifloxacin dose was 20 mg/kg/day, with neither micronuclei or significant bone marrow toxicity observed. Human studies (both sexes) using repeated oral gemifloxacin daily doses of 320 mg/day have demonstrated an average Cmax of approximately 1.5 g/ml (range from 0.7-2.6 g/ml) and average AUC of about 9 gh/ml (range from 4.7-20.1 gh/ml). As has been discussed in the previous report, however, it is probably not appropriate to use either Cmax or AUC by itself to predict *in vivo* micronucleus formation in bone marrow PCEs (see report directly above).

SPECIAL TOXICOLOGY STUDIES

Generation of Anti SB-265805-S Antisera in the Guinea Pig (Study Code SBF 236/993140; SB Document No. SB-265805/RSD-10110F/2)

NDA 21,158-000/Factive (gemifloxacin)

Report issued 8/6/99, amended 10/20/99, —— and —— GLP

Vol. 1.5 005

Animals: Male Dunkin Hartley guinea pigs, approximately weeks old 372-410 g at the start of the study, housed in groups of 5, 5 per test group for challenge and 5 per test group for antibodies

Diet: ——————————Standard guinea pig diet and tap water were provided *ad libitum*.

Study Conduct: Guinea pigs were sensitized to gemifloxacin (Batch No. EF03-12R1P5) alone (6.67 mg/kg) or gemifloxacin conjugated to human serum albumin (HSA) (10 mg/kg as conjugate). Test articles were dissolved in 0.9% saline and given weekly for 4 weeks by the subcutaneous (SC) route at a dose volume of 3 ml/kg. Freund's complete adjuvant was used for the first dose, with Freund's incomplete adjuvant used for the next 3 doses (FA). A negative control group received saline with FA.

The animals in the antibody collection group were bled 7 days after the last sensitization dose. The antigen challenge was given to the guinea pigs assigned to that study group 15 days after the last sensitization dose. The challenge antigen was gemifloxacin conjugated to guinea pig serum albumin and it was given IV at a dose of 10 mg/kg (based on the conjugate) with a dose volume of 1 ml/kg. Guinea pigs were observed continuously for 30 minutes after the challenge dose, then intermittently for 3 hours more. Naïve animals were also given IV doses of the challenge antigen to rule out an anaphylactoid reaction. Anaphylaxis was scored as follows:

0	Normal
+	Piloerection, nose scratching, and unrest
++	Above signs, plus tremors and sneezing
+++	Above signs, plus urination, defecation, dyspnea, and ataxia
++++	Above signs plus convulsion
+++++	Death

Results: None of the animals that were sensitized subcutaneously with vehicle or 6.67 mg/kg of gemifloxacin alone demonstrated signs of anaphylaxis (0/5 for both groups). The guinea pigs sensitized with 10 mg/kg gemifloxacin conjugated with human serum albumin all responded to the antigen challenge with active anaphylaxis (5/5). The severity of the responses ranged from ++ to death (+++++).

The report contained no data regarding antibody production. The serum specimens from the animals assigned to the antibody group were sent to the sponsor for evaluation and the results were to have been reported separately. A separate report describing these results has not yet been submitted.

NDA 21,158-000/Factive (gemifloxacin)

SB 265805: 13-Week Oral (Gavage) Subchronic Study in Hairless Mice, With or Without Added Simulated Sunlight (SB Document No. SB-265805/RSD-10110V/1; Protocol No. G98024)

Study Report: [redacted]
Pathology Report: [redacted]

Study Report dated 10/15/99, U.S. GLP
Pathology Report dated 6/10/99

Vol. 1.5.018

Animals: Crl:SKH1-*hr*BR hairless mice, 88 days old and 28-42 g (males) or 23-32 g (females) at the initiation of the study. Gold fluorescent lighting [redacted] was used in the rooms where mice were housed and exposed to UV to prevent the animals from being exposed to light radiation that could interfere with the study. Each treatment group had 6 mice/sex.

Diet: Certified Rodent Diet [redacted] and tap water purified via reverse osmosis and chlorinated were available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin mesylate (Batch No: 03R1P2-1-1(3)) was dissolved in 0.9% saline and administered orally once per day at doses of 25, 50, 100, or 200 mg/kg (based on free drug). Lomefloxacin (20 and 100 mg/kg in 0.9% saline) was used as a reference compound. Negative controls consisted of vehicle and untreated mice. The dose volume was 10 ml/kg for all test materials.

The doses of drugs were chosen based upon the results of a range-finding study where hairless mice were given single doses of gemifloxacin (50, 100, 200, or 400 mg/kg), lomefloxacin (20 or 200 mg/kg), ciprofloxacin (100 or 400 mg/kg), or vehicles (0.9% saline or 0.5% methylcellulose), then exposed to simulated sunlight (0.25-1.4 times the estimated minimal erythemal dose, MED) and observed for 72 hours. Mortality was observed in the 200 and 400 mg/kg gemifloxacin groups (1/6 and 2/6 mice) and the 200 mg/kg lomefloxacin group (1/6). Phototoxicity was not observed at any dose of gemifloxacin, but was seen after 200 mg/kg lomefloxacin or 400 mg/kg ciprofloxacin.

Length and Conduct of Study: Test substances were given 5 days per week for up to 13 weeks. UV exposure occurred one hour after dosing on Monday, Wednesday, and Friday, and one hour before dosing on Tuesday and Thursday. There were 2 groups for each treatment described above with one of the two groups receiving 600 Robertson-Berger Units (RBU; 400 RBU is approximately one MED in untanned human skin) of UV per week and the others receiving no UV exposure. The UV source was a [redacted] UV exposure was

monitored throughout the study using detectors mounted on animal racks.

The mice were observed twice daily for viability and once weekly for skin reactions. Body weights were measured weekly. Skinfold thickness was measured at 3 sites on the rump of each mouse during weeks 8 and 13 of treatment. Mice were sacrificed on day 95 of the study and a 3 cm x 3 cm area of dorsal skin was harvested for microscopic examination. The livers and kidneys of all mice were also examined histologically. Tissues were preserved in 10% neutral buffered formalin and sent to [redacted] for analysis.

Results: In the 200 mg/kg gemifloxacin group, 2 males (one each in the UV and no UV groups) and 1 female (UV group) were found dead during week 1. Clinical signs in the surviving mice from this group included decreased motor activity, impaired righting reflex, bradypnea, and scant feces. The remaining mice in the 200 mg/kg gemifloxacin groups were sacrificed on days 4 and 5 of the study due to the mortality and clinical signs observed in this group. Necropsy revealed obstructive tubular nephropathy of varying severity in most of these mice (multifocal tubular degeneration and/or dilation and with crystals in the papillary collecting ducts).

One mouse each in the vehicle + UV group (female), 25 mg/kg gemifloxacin + UV group (male) and 100 mg/kg gemifloxacin no UV group (male) died as the result of gavage errors.

Treatment-related changes in body weight were not observed during this study.

Signs of phototoxicity (erythema, edema and flaking of skin) were observed in the 100 mg/kg lomefloxacin + UV group. In week 4, grade 1 edema (mild, raised <1 mm) was observed in 5/6 male and 5/6 female mice and grade 1 erythema (barely perceptible light redness) was seen in 1/6 male and 2/6 female mice. Flaking (also grade 1; barely perceptible scales) began to be seen during week 4 in females (2/6) and during week 5 in males (3/6). Severity of these phototoxic signs did not increase over the course of the study, but the incidence of the effects generally did. During week 13, 6/6 males in the 100 mg/kg lomefloxacin + UV group had Grade 1 erythema and edema and 1/6 had Grade 1 flaking. At the same observation time, 6/6 females had Grade 1 erythema, 4/6 had Grade 1 edema, and 2/6 had Grade 1 flaking. (The peak incidence of flaking in females was 5/6 during week 11.) At the final observation point (considered week 14 in the report), 5/6 males had Grade 1 erythema and edema and 2/6 males had Grade 1 flaking. In females, 6/6 had Grade 1 erythema, 5/6 had Grade 1 edema, and 1/6 had grade 1 flaking. Macroscopic and histologic examination of the skin from the mice in the 100 mg/kg lomefloxacin + UV group revealed mild to marked acanthosis (12/12), dermal abscess (1/12), sebaceous gland hyperplasia (6/12), microgranulomas (2/12), and dermal inflammation (5/12).

Signs of phototoxicity were not seen in the 20 mg/kg lomefloxacin + UV group, the vehicle + UV group, or in any of the gemifloxacin + UV groups. Signs of phototoxicity were not observed in groups not exposed to UV. Minimal or mild acanthosis was observed in some mice from all of these treatment groups; this was observed with greater frequency in the mice exposed to UV (including controls) than in those not exposed to UV.

Mean skinfold thickness in unirradiated male and female mice was similar across all dose groups. In the 100 mg/kg lomefloxacin + UV group (both males and females), mean skinfold thickness was significantly greater ($p \leq 0.05$ or 0.01) than controls (untreated or vehicle treated mice exposed to UV) during both weeks 8 and 13. Mean skinfold thickness was not increased in any of the gemifloxacin + UV treatment groups, nor was it increased in the 20 mg/kg lomefloxacin + UV group.

Microscopic examination did not reveal any gemifloxacin-related changes in the liver, but tubular nephropathy was seen in one of the 100 mg/kg male mice.

Under the conditions of this study, gemifloxacin was not associated with phototoxicity in hairless mice at doses up to 100 mg/kg (13 weeks of drug + UV). A higher dose (200 mg/kg) of gemifloxacin was not tolerated due to obstructive tubular nephropathy. This nephropathy was also observed in one male mouse from the 100 mg/kg gemifloxacin group.

SB 265805: Oral (Gavage) Toxicokinetic Study in Hairless Mice (SB Document No. SB-265805/RSD-100ZTZ/2; SB Study No. G98070; —— Protocol No. 2619-035)

Report dated 3/23/99, amended 10/15/99; Signed QA statement present

Vol. 1.5.019

Animals: Female Crl:SKH1-*hr*BR hairless mice, approximately 16 weeks old and 24-33 g at the beginning of the study, 3 per time point for each dose group

Diet: Certified Rodent Diet —— and tap water purified via reverse osmosis and chlorinated were available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin mesylate (Batch No. 03R1P2-1-1(3)) was dissolved in 0.9% saline and administered once via oral gavage (10 ml/kg) at doses of 25, 50, 100, or 200 mg/kg (based on free drug).

Length and Conduct of Study: Blood samples were drawn (into EDTA) from the mice (3 per dose group at each time point) 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after dosing. Plasma was frozen and sent to SmithKline Beecham from —— for analysis using a —— procedure with a lower limit of quantification of — ng/ml. The SKB analysis of the samples is described in an appendix ——— to the study report.

Results: No unscheduled deaths occurred and no clinical signs of drug toxicity were reported. Maximum plasma concentrations for each dose were achieved 0.5 hours after dosing (the first sampling time), so they may have been underestimated. Plasma levels of

gemifloxacin could be measured up to 6 hours after dosing at 25 mg/kg and for at least 8 hours after dosing in the rest of the groups. Accumulation of gemifloxacin in this species would not be expected since it was not quantifiable in plasma beyond 12 hours after dosing. Cmax was dose-proportional up to 100 mg/kg, but it did not increase between 100 and 200 mg/kg. AUC increased over the entire dose range in a generally dose-proportional manner.

Composite Toxicokinetic Parameters for Gemifloxacin Following a Single Oral Administration to Hairless Mice

Dose (mg/kg)	Cmax (g/ml)	AUC_{0-t}(gh/ml)
25	0.299	0.481
50	0.430	1.01
100	3.23	3.47
200	3.09	6.83

SB 265805: Four-Week Oral (Gavage) Toxicokinetics Study in Hairless Mice (SB Document No. SB-265805/RSD-10110N/1; SB Study No. G98178; ——— Protocol No. 2619-038)

———————————————

Report dated 10/20/99, U.S. GLP

Vol. 1.5.019

Animals: Crl:SKH1-*hr*BR hairless mice, approximately 9 weeks old, 23-34 g (males) and 19-28 g (females) at the initiation of dosing, 3/sex per time point for each dose group

Diet: Certified Rodent Diet ——————————and tap water purified via reverse osmosis and chlorinated were available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin mesylate (Lot No. FSN-A-05C(1)) was dissolved in 0.9% saline and administered via oral gavage (10 ml/kg) at doses of 10, 50, or 100 mg/kg (based on free drug). The drug was given to groups of mice as a single dose without irradiation, or 5 days per week for 4 weeks with exposure to simulated sunlight. Lomefloxacin (also dissolved in 0.9% saline) was given to a group of mice at 100 mg/kg/day, 5 days a week for 4 weeks with irradiation. This study was performed to support the photocarcinogenicity study below.

Length and Conduct of Study: For the mice that were used in the multiple dose portion of the study, test substances were given 5 days per week for 26 days. UV exposure occurred one hour after dosing on Monday, Wednesday, and Friday, and one hour before dosing on Tuesday and Thursday. The mice received 600 Robertson-Berger Units (RBU; 400 RBU is approximately one MED in untanned human skin) of UV per week. The UV source was a [redacted] UV exposure was monitored throughout the study using detectors mounted on animal racks. The mice used for the single dose portion of the study were not exposed to UVR.

Blood samples were drawn (into EDTA) from the mice (3/sex per dose group at each time point) 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after dosing (after the single dose or following the day 26 dose, as appropriate). Skin samples (3 cm X 3 cm from the dorsum and sides of the animal) were taken immediately after the blood was drawn. Plasma and skin were frozen and sent to [redacted], for chloroform extraction and analysis using a validated [redacted] method with [redacted] detection with lower limits of gemifloxacin quantification of [redacted] ng/ml in plasma and ng/g in skin and lower limits of lomefloxacin quantification of [redacted] ng/ml in plasma and ng/g in skin. The [redacted] analysis of the samples is described in an appendix [redacted] to the study report.

The mice were observed twice daily for viability and once weekly for skin reactions. Body weights were measured weekly.

Results: No unscheduled deaths or clinical signs of toxicity were reported. Treatment-related effects on body weights were not observed. No skin reactions were seen on days 0, 7 and 14 of the study. The reactions reported on days 21 and 26 (erythema, edema, flaking) could not be assessed for drug enhancement of UV exposure effects because there were no control groups exposed to simulated sunlight in this study.

Tmax for both skin and plasma was between 0.5 and 1 hour for gemifloxacin and lomefloxacin. Increases in Cmax and AUC were not always perfectly dose-proportional, but generally tended to be dose-proportional. Cmax and AUC values for skin were higher than those for plasma for both drugs. In skin, and to a lesser degree, in plasma, Cmax and AUC values were higher on day 26 then on day 1 in the 100 mg/kg gemifloxacin dose group, but not the 2 lower dose groups. There did not appear to be gender differences in the toxicokinetic parameters for these drugs. Lomefloxacin plasma and skin concentrations were higher than those achieved for the same nominal dose (100 mg/kg) of gemifloxacin.

Composite Toxicokinetic Parameters for Gemifloxacin and Lomefloxacin in Plasma Following Oral Administration to Hairless Mice

Dose (mg/kg)	Males		Females	
	Cmax (g/ml)	AUC_{0-t}(gh/ml)	Cmax (g/ml)	AUC_{0-t}(gh/ml)

	Day 1	Day 26	Day 1	Day 26	Day 1	Day 26	Day 1	Day 26
Gemifloxacin								
10	0.155	0.074	0.228	0.173	0.133	0.098	0.250	0.213
50	0.576	0.366	1.123	0.741	0.998	0.348	1.139	0.791
100	0.746	1.529	2.107	2.840	0.953	1.298	2.811	3.414
Lomefloxacin								
100	9.903	7.227	20.062	19.423	8.109	7.311	19.204	19.564

Composite Toxicokinetic Parameters for Gemifloxacin and Lomefloxacin in Skin Following Oral Administration to Hairless Mice

Dose (mg/kg)	Males				Females			
	Cmax (g/g)		AUC_{0-t}(gh/g)		Cmax (g/g)		AUC_{0-t}(gh/g)	
	Day 1	Day 26	Day 1	Day 26	Day 1	Day 26	Day 1	Day 26
Gemifloxacin								
10	0.491	0.347	1.709	1.586	0.628	0.571	1.814	2.282
50	3.684	1.624	6.896	5.528	3.811	2.014	7.932	7.885
100	4.706	7.483	11.826	17.213	4.715	7.320	14.375	25.103
Lomefloxacin								
100	20.456	16.511	48.514	56.910	18.403	20.783	52.347	71.508

SB 265805: 12-Month Oral (Gavage) Study to Determine the Influence on Photocarcinogenesis in Hairless Mice (SB Document No. SB-265805/RSD-1014WP/1; SB Study No. G98092; [redacted] Protocol No. 2619-036)

Report dated 10/20/99, U.S. GLP

Vols. 1.5.020 and 1.5.021

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Animals: Crl:SKH1-*hr*BR hairless mice, approximately 8 weeks old, 24-35 g (males) and 19-28 g (females) at the time of study assignment, 34/sex for each dose group. Gold fluorescent lighting [redacted] was used in the rooms where mice were housed and exposed to UV to prevent the animals from being exposed to light radiation that could interfere with the study.

Diet: Certified Rodent Diet [redacted] and tap water purified via reverse osmosis and chlorinated were available *ad libitum*.

Drug Dose and Route of Administration: Gemifloxacin mesylate (Lot No. FNS-A-

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05C(1)).was dissolved in 0.9% saline and administered via oral gavage (10 ml/kg) at doses of 10, 50, or 100 mg/kg (based on free drug). Lomefloxacin (also dissolved in 0.9% saline) was given to a group of mice at 100 mg/kg/day. One group of mice received vehicle only and 2 other groups received only UVR without any drug or vehicle. Doses were chosen based upon the results of a 13 week study in the same species of mouse. A 200 mg/kg dose of gemifloxacin was not tolerated by the mice due to obstructive renal tubular nephropathy secondary to crystal formation.

Length and Conduct of Study: Test substances were given 5 days per week for 40 weeks. UV exposure occurred one hour after dosing on Monday, Wednesday, and Friday, and one hour before dosing on Tuesday and Thursday. All groups of mice but one undosed control received 600 Robertson-Berger Units (RBU; 400 RBU is approximately one MED in untanned human skin) of UV per week. The other undosed control received a higher exposure to UVR, 1200 RBU per week. [redacted] UV exposure was monitored throughout the study using detectors mounted on animal racks. The mice were observed for an additional 12 weeks without drug or UVR treatment, then sacrificed and examined for gross lesions.

The mice were observed twice daily for viability and once weekly for skin reactions. Body weights were measured weekly for the first 13 weeks of treatment, then every 4 weeks until sacrifice. Mice with a tumor >10 mm in planar diameter were sacrificed and an entire group of mice would be sacrificed when fewer than one half survived and more than half of the survivors had tumors at least 4 mm in planar diameter. Treatment groups were as follows:

1. Vehicle	600 RBU/week
2. 10 mg/kg gemifloxacin	600 RBU/week
3. 50 mg/kg gemifloxacin	600 RBU/week
4. 100 mg/kg gemifloxacin	600 RBU/week
5. No drug or vehicle	600 RBU/week
6. No drug or vehicle	1200 RBU/week
7. 100 mg/kg lomefloxacin	600 RBU/week

Results: Treatment of hairless mice with up to 100 mg/kg/day of gemifloxacin plus simulated sunlight did not directly affect survival compared to vehicle control. Survival was >90% in the vehicle control, undosed control and gemifloxacin treated groups exposed to simulated sunlight (600 RBU/week) through week 38. None of the unscheduled deaths (generally 1-3 per sex in each dose group, unrelated to tumor burden) that occurred during the study appeared to be related to drug treatment. Survival in the undosed high UV (1200 RBU/week) and the lomefloxacin plus simulated sunlight groups rapidly declined after weeks 28-29 because mice were being sacrificed due to tumor burden.

The doses of gemifloxacin used in this study did not have a biologically

significant effect on mean body weights. None of the in-life or necropsy observations (excluding skin reactions) were associated with a specific treatment. Skin reactions that were observed in the vehicle control, undosed control (low UV) and gemifloxacin groups included erythema, edema, flaking, thickening, and ulcerations. The skin reactions among these groups tended to be similar and were likely secondary to the simulated sunlight exposure (600 RBU/week). The skin reactions seen in the gemifloxacin-treated mice were not more severe than those observed in the vehicle controls. The incidence and severity of erythema and edema were greater in the undosed control high UV (1200 RBU/week) and lomefloxacin groups than in the others and the incidence and severity of flaking was greater in the undosed control high UV group than in the rest.

As can be seen in the table below, oral administration of up to 100 mg/kg of gemifloxacin did not alter the median time to ≥1 mm tumor onset compared to mice not treated with drug when both were exposed to low dose UV (600 RBU/week). Exposure of the hairless mice to high UV or lomefloxacin plus low UV significantly decreased the median onset time until tumors ≥1mm were observed. Tumor response patterns in male and female mice were similar, so both genders have been combined for presentation of data. Tumor yield (number of tumors per surviving mouse) was greater in the high UV mice and the lomefloxacin-treated mice compared to any of the low UV groups (including undosed, vehicle, and gemifloxacin-treated).

Development of Tumors (≥1 mm) in Mice Exposed to Vehicle or Gemifloxacin and Simulated Sunlight

Drug Treatment (mg/kg)	Unbiased* Median Tumor (≥1 mm) Onset [Upper and Lower 95% Confidence Limits]	Tumor (≥1 mm) Yield at Week 31** [Number of Surviving Mice]
Vehicle Control	36 weeks [35, 37]	0.25 [65]
Gemifloxacin, 10 mg/kg	38 weeks [36, 40]	0.07 [67]
Gemifloxacin, 50 mg/kg	39 weeks [37, 46]	0.09 [65]
Gemifloxacin, 100 mg/kg	39 weeks [37, 46]	0.08 [65]
Undosed Low UV Control	39 weeks [37, 41]	0.08 [66]
Undosed High UV Control	25 weeks [24, 26]***	3.53 [57]

Lomefloxacin, 100 mg/kg	23.5 weeks [22, 26]***	4.92 [53]

*Unbiased data analysis took into account the tumor latency periods of mice that died without developing tumors.

**Week 31 was chosen because it was the final week where similar numbers of mice remained in each group. After this time, more mice in the high UV group began to be sacrificed after having met the tumor burden criteria defined in the protocol. The week before the vehicle control group was sacrificed upon having met the tumor burden criteria (week 48), tumor burden was not significantly greater in the gemifloxacin-treated mice than in the vehicle controls (8.74, n=38), 10 mg/kg gemifloxacin (7.47, n=47), 50 mg/kg gemifloxacin (6.49, n=51), 100 mg/kg gemifloxacin (5.85, n=41) or undosed low UV (5.42, n=50) treatment groups.

***$p<0.001$ compared to Vehicle Control (using Peto Analysis, 2-tailed p value)

Gemifloxacin, at doses of up to 100 mg/kg, did not shorten the time to observation of UV-induced tumors in hairless mice or increase their number or severity. The positive control substance, lomefloxacin, clearly reduced the time until observation of UV-induced tumors and increased tumor yield in the mice. The concentration of gemifloxacin in the skin of the mice at Cmax, around the time of irradiation (on the days when drug was given prior to UV exposure), following a 100 mg/kg dose was about 6.06 g/g when the skin data from the preceding supportive toxicokinetic study were averaged. Plasma levels following a 100 mg/kg dose were about 1.13 g/ml in the mice around the time of irradiation. There are no data on gemifloxacin skin levels in humans, but the mouse plasma gemifloxacin levels are in the expected range of human plasma Cmax levels (0.7-2.6 g/ml, with overall mean of about 1.5) following multiple 320 mg oral doses. Gemifloxacin was not photocarcinogenic in the hairless mouse model.

Microscopic Examination of Liver From DMPK Study No. 6146-205: "Biliary and Plasma Concentrations of Drug-Related Material Following Single and 5-Day Repeat Administration of [14C]SB-265805-S to Male Bile Duct-Cannulated Beagle Dogs at a Target Dose of 30 mg Free Base/kg/day (SB Document No. SB-265805/RSD-1014X3/1; Protocol No. I99620)

D. Brees, A. Pritchard (SmithKline Beecham, The Frythe, Welwyn, Herts, UK)

Report dated 10/28/99

Vol. 1.5.022

NDA 21,158-000/Factive (gemifloxacin)

Summary: The liver samples examined microscopically were from a PK study in which 3 bile duct cannulated male beagle dogs received 30 mg/kg/day of IV gemifloxacin (^{14}C-labeled) for 5 days via slow bolus injection at a dose volume of 5 ml/kg. The dogs were sacrificed about 6 hours after the final dose of drug was administered. The microscopic examination of the tissue included observation under polarized light to enhance the detection of crystals. Peer review of the histopathology occurred.

Crystals were observed in the bile ducts in all dogs. The amount of crystal formation in the bile ducts was mild to moderate in one dog, moderate in a second dog, and minimal (seen only in one bile duct) in the third. Cholangitis/pericholangitis and hepatocellular degeneration/
necrosis correlated directly with the bile duct crystal formation and were observed mainly in periportal areas. Crystals were occasionally associated with hepatocytes or were seen within hepatocytes. Mild crystal formation in bile duct canaliculi in the periportal regions was observed in all of the dogs and these were often associated with brown pigment (believed to be evidence of intrahepatic cholestasis).

Microscopic Findings in Dog Liver Specimens

	Dog H04328	**Dog H04330**	**Dog H04331**
Crystals in Bile Ducts	Mild to moderate	Minimal- only in one bile duct	Moderate
Cholangitis/Pericholangitis	Mild	Not Present	Mild to Moderate
Hepatocellular Degeneration Necrosis	Mild	Minimal	Mild

These findings provide evidence that gemifloxacin hepatotoxicity in dogs is associated with crystal formation and deposition in the biliary tree of the liver.

X-Ray Energy Spectroscopy of Bile Duct Inclusions from a Dog Dosed with SB-265805-S (I99544) (SB Document No. SB-265805/RSD-1014XF/1)

Report dated 10/25/99
Vol. 1.5.022

Summary: Liver sections from a control dog and a dog given 30 mg/kg IV gemifloxacin for 4 days (from the study conducted under Protocol D97671) were examined by scanning electron microscopy. An X-ray energy spectrometer ——— was used to examine the crystals, other areas of the hepatocytes with no crystals and areas of the slide with no tissue (for comparative purposes).

No crystals were present in the specimens from the control dog. Three bile duct

crystals were analyzed from the specimen of liver from the gemifloxacin-treated dog. Magnesium and fluorine were associated with the crystals, but not with areas of the same specimen where no crystals were present. Gemifloxacin contains fluorine and fluoroquinolones are known to chelate magnesium. X-ray analysis of the control tissue showed similar spectra (carbon, oxygen, phosphorus, sulfur, chlorine- substances associated with biological specimens) as were observed in the areas of the liver tissue of the gemifloxacin-treated dog that did not have crystals.

These data suggest that the crystals in the bile duct of the gemifloxacin-treated dog were gemifloxacin (and/or a metabolite) in a complex with magnesium.

Solubility of SB-265805 (free base) in Gallbladder and Hepatic Dog Bile (SB-265805/RSD-1014S7/2)

Report dated 9/24/99, amended 11/3/99

Vol. 1.5.022

Summary: Gallbladder bile was obtained from one 14 month male beagle dog. Three aliquots from this sample were tested for pH and solubility. Hepatic bile was collected from 3 male beagle dogs; two aliquots of each sample were tested. To test solubility, 6-7 mg of gemifloxacin free base were mixed with 0.3 ml of dog bile and rotated in a 37C incubator for 30 minutes. Some incubations went for 60 minutes to confirm that 30 minutes was long enough to achieve maximum solubility. After incubation, each aliquot of bile was centrifuged and filtered (0.45 m pore size) to remove undissolved drug. Samples underwent [redacted] and [redacted] with [redacted] detection was used to measure the amount of gemifloxacin (LB20326a was used as an internal standard).

The mean pH (at room temperature) of the gallbladder bile was 7.12 ± 0.09 and of the hepatic bile was 8.44 ± 0.12. The mean solubility of gemifloxacin free base in gallbladder bile was 1.70 ± 0.06 mg/ml and in hepatic bile it was 0.60 ± 0.13 mg/ml.

A previous study conducted by the original developer of gemifloxacin (LG Chemicals, Korea) had demonstrated that this drug was more soluble in dog bile (5.08 mg/ml) than in rat or human bile (0.87 and 1.21 mg/ml, respectively). The source (gallbladder or liver) and pH of the bile specimens used in the older study were not specified (though SKB believed it was probably gallbladder bile), so the current study was undertaken by SKB to confirm their hypothesis that gemifloxacin may be less soluble in hepatic bile vs. gallbladder bile from dogs since crystals associated with the bile ducts were frequently being observed in the livers of these animals.

The results of this study indicate that gemifloxacin is more soluble in dog bile from the gallbladder than hepatic dog bile, with the pH difference (pH is higher in hepatic bile) a factor in the differential solubility.

NDA 21,158-000/Factive (gemifloxacin)

RECOMMENDATIONS FOR LABEL: The reviewer's suggested deletions from the sponsor's proposal are struck out and suggested additions are in ***bold italic*** text. Additionally, the statement that "Nearly all fluoroquinolones... have been shown to cause prolongation of the QT interval" (see page 13 of the original label proposal from the sponsor) is questionable. The reviewer suggests modifying this to say "several" or "a number of" fluoroquinolones... have been shown... Under Information for Patients, paragraph 3 on page 14, add "(e.g., tanning beds)" after the phrase "artificial ultraviolet light."

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis: *Long term studies in animals to determine the carcinogenic potential of gemifloxacin have not been conducted.*

Photocarcinogenesis:

[redacted]

Mutagenesis:

[redacted]

[

]

Impairment of Fertility:

[

]

Pregnancy: Teratogenic Effects. Pregnancy Category C.

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Nursing Mothers

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OVERDOSAGE

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NDA 21,158-000/Factive (gemifloxacin)

OVERALL SUMMARY AND EVALUATION:

A comprehensive review of the nonclinical pharmacokinetics and metabolism of gemifloxacin has been written by Dr. Stephen Hundley, and the reader is referred to that document for details on this topic. Gemifloxacin is rapidly absorbed from the GI tract of rats and beagle dogs following oral administration. Studies in rats using radiolabeled gemifloxacin demonstrated extensive tissue distribution following oral dosing. Most tissues had higher levels of the drug than plasma with the exception that fat and brain levels of gemifloxacin were lower (about 1/3 and 1/10 of the plasma levels, respectively). Plasma protein binding of gemifloxacin *in vitro* in the mouse, dog, rat, and human was moderate, ranging from 57-75%. Like other quinolones, gemifloxacin appears to have a high affinity for melanin. Elimination of radiolabeled drug from the uveal tract of the eye in a pigmented rat was much slower than elimination from other tissues that did not contain melanin. Pharmacokinetics of the 2 enantiomers present in gemifloxacin do not differ significantly. The half life of gemifloxacin in rats is approximately 2 hours and in dogs it is about 5-6 hours. The volume of distribution in dogs is 5 L/kg at steady state and clearance is 0.8 L/hr/kg; these parameters in rats are approximately 4.3 L/kg and 2.4 L/hr/kg, respectively. The oral bioavailability of gemifloxacin was difficult to determine definitively. Data from bile duct cannulated rats and dogs that received radiolabeled drug IV indicated that gemifloxacin and/or its metabolites are actively secreted from the intestinal epithelium into the lumen of the GI tract and excreted in the feces. Following IV administration of 10 mg/kg gemifloxacin to bile duct cannulated rats, 40% of the dose was excreted into the urine, 30% in feces and 12% in bile over a 24 hour period. Unchanged drug made up 60-70% of the urinary excretory products for gemifloxacin and urinary metabolites included the E-isomer of gemifloxacin (7-9%), N-acetyl gemifloxacin (2-4%), an acyl glucuronide, and glutamic acid conjugates of the parent compound and the N-acetyl metabolite (all $\leq$ than 2%). Gemifloxacin acyl glucuronide was the major component of the bile (32-38% of drug associated material in bile), with 15-16% unchanged drug present. Other biliary metabolites in the rat were the gemifloxacin E-isomer (1%) and its acyl glucuronide (3-4%), N-acetyl gemifloxacin (6-7%) and its acyl glucuronide (1-2%), the glutamic acid conjugate of gemifloxacin (3-4%), and deaminated gemifloxacin (1-3%) and its acyl glucuronide (3-7%). In the feces of rats, unchanged drug accounted for 50-70% of excreted material and metabolites included the E-isomer (3-4%), N-acetyl gemifloxacin (1-7%) and its acyl glucuronide (1%), and deaminated gemifloxacin (2%). When 10 mg/kg gemifloxacin was given IV to bile duct cannulated dogs, 27% of the dose was recovered in the urine, 39% in feces, and 30% in bile (over a 24-48 hour period). As in rats, the parent compound was the predominant gemifloxacin species found in the urine (73-80%). The E-isomer (6-11%), acyl glucuronide (3-5%), and O-desmethyl gemifloxacin (1-3%) were other urinary metabolites. The biliary excretory products in the dog were unchanged drug (34%), gemifloxacin acyl glucuronide (20-22%), O-desmethyl gemifloxacin (6-8%), carboxylic acid of gemifloxacin (5-7%), and 5% or less of gemifloxacin acyl glucoside, deaminated gemifloxacin and its acyl glucuronide, and the E-isomer of gemifloxacin. In feces from dogs, only unchanged drug (40-70%, the E-isomer (3-6%), and deaminated gemifloxacin

(1%) were detected. As the gemifloxacin acyl glucuronide was not found in the feces of either rats or dogs, but had been a major biliary excretory product in both species, the gut bacteria are likely to have hydrolyzed this conjugate.

Mortality occurred following single oral gemifloxacin doses of 2000 mg/kg in rats and 400 mg/kg in mice. The minimum lethal intravenous doses in these species were 200 and 100 mg/kg, respectively. Toxic signs after administration of a single high dose of gemifloxacin to rodents included ataxia, lethargy, piloerection, cyanosis, tremor, and clonic convulsions.

When gemifloxacin was administered orally once daily to rats for 7 days at 1000 mg/kg, crystal nephropathy was observed in all animals. Drug-related crystal nephropathy has been seen in rats when other quinolones were administered. Reduced food consumption was observed in both genders at this dose, and a slight reduction in hemoglobin concentration, red blood cell count, and packed cell volume was observed in the females. The NOAEL for gemifloxacin was 250 mg/kg in this study.

Oral administration of gemifloxacin to rats at doses up to 750 mg/kg for 28 days was not associated with any overt clinical signs of toxicity. Cecal dilatation, a common finding in rats treated with antimicrobials, was observed in a few rats across all gemifloxacin dose groups. Microscopic examination of the kidneys demonstrated crystal nephropathy in 1/10 60 mg/kg males, 5/10 210 mg/kg males, 10/10 750 mg/kg males and 6/10 750 mg/kg females. One high dose female had cholangitis/pericholangitis in its liver. The NOAEL in female rats was 210 mg/kg/day and in males, it was less than 60 mg/kg/day after 28 days of administration.

Crystal nephropathy was observed in male rats given daily oral gemifloxacin doses of 90 or 210 mg/kg for 13 weeks. Increased kidney weights and changes in serum chemistry consistent with renal toxicity were seen these rats. Histopathological changes in the kidney were not observed in males given 30 mg/kg of gemifloxacin or in females from any dose group. With the exception of increased water consumption in the drug-treated rats, no drug-related clinical signs or effects on food intake were noted.

Gemifloxacin was associated with a dose-related decrease in food consumption and body weight loss when administered IV to rats at 60 or 100 mg/kg/day for 4 days. Drug-related histopathologic changes included hypocellularity of bone marrow in the sternum (associated with decreased reticulocytes and white blood cells in the peripheral blood), renal tubular nephropathy (probably related to drug-associated material found in the kidney and associated with increased plasma urea and creatinine levels), and pericholangitis (possibly related to plugs in the bile ducts that may contain drug-associated material). Bruising and necrosis of the injection site was observed as early as day 2 of dosing in the 100 mg/kg rats and led to some animals being sacrificed early for humane reasons. Excluding one male with an injection site lesion, the NOAEL for gemifloxacin administered IV to rats daily for 4 days was 10 mg/kg.

In an IV rat study where gemifloxacin was given for 14 days, approximately half of the animals given 40 mg/kg/day of gemifloxacin and one given 20 mg/kg/day via the tail vein had to be sacrificed before the end of the study period due to the poor condition of their tails. The injection sites of many rats in these dose groups (including premature

decedents and survivors) were discolored, with inflammatory cell infiltration and epidermal/dermal necrosis observed microscopically. Decreased food consumption and body weight gain were seen during the first week of dosing with 40 mg/kg of gemifloxacin. Increased water consumption was seen at 40 mg/kg and signs of a slightly increased plasma volume were observed in the rats from this dose group (slight decreases in red cell parameters and plasma albumin concentration). A smaller increase in water consumption was also observed at 20 mg/kg. In contrast to the previous 4 day IV study in rats (which used doses as high as 60 and 100 mg/kg/day) hypocellularity of bone marrow in the sternum (associated with decreased reticulocytes and white blood cells in the peripheral blood) and pericholangitis were not observed in the current 14 day study. Renal tubular nephropathy was observed in 1/10 rats in the 10 mg/kg group, 7/10 at 20 mg/kg, and all 40 mg/kg rats. Microscopic changes in the kidneys included basophilia of the tubular epithelial epithelium of the distal nephron, tubular dilatation, and interstitial inflammatory cell infiltration. Plugs in the distal tubules of rats from the 20 and 40 mg/kg dose groups appeared similar to those seen in the previous 4 day study that were shown to contain drug-related material. Changes in serum chemistry in the 40 mg/kg group (increased urea and creatinine levels) and decreased urinary creatinine clearance were also indicative of renal toxicity. The NOAEL for gemifloxacin administered IV daily for 14 days was 2 mg/kg.

Intravenous infusion of rats with 10 or 20 mg/kg/day of gemifloxacin for 28 days via an indwelling catheter (the other rat IV studies used a slow bolus injection technique) was associated with an increased incidence of thrombi and increased severity of injection site reactions compared to controls. The severity of the injection site reactions was associated with crystal deposition. Decreased food consumption and body weight gain were seen during the first several days of dosing with 20 mg/kg of gemifloxacin, but both were similar to controls for the rest of the treatment period. Increased water consumption was seen in males at 20 mg/kg and signs of a slightly increased plasma volume were also observed in the rats from this dose group (slight decreases in red cell parameters and plasma protein and globulin concentrations). These sort of hematologic changes have been observed in previous studies with rats, they were no longer evident after the 4 week recovery period and their magnitude was too small for them to be of biological significance. As in the 14 day IV study in rats discussed above, hypocellularity of bone marrow in the sternum, decreased reticulocytes and white blood cell counts, and pericholangitis were not observed. Renal tubular nephropathy was observed in 10/10 males and 1/10 females from the 20 mg/kg group and 4/10 males in the 10 mg/kg group. Microscopic changes in the kidneys included basophilia of the tubular epithelium, tubular dilatation, interstitial inflammatory cell infiltration, and papillary epithelial hyperplasia. Plugs in the distal tubules and/or renal pelvis of rats from the 10 and 20 mg/kg dose groups appeared similar to those seen in the previous studies that were shown to contain drug-related material. The NOAEL for gemifloxacin administered IV daily for 28 days to rats was 2 mg/kg.

After 28 days of oral gemifloxacin administration to dogs, the NOAEL was 120 mg/kg. One female in this group had a slight increase in ALT, but microscopic changes in its liver were minimal and similar findings were observed in a few control dogs. A

360 mg/kg dose exceeded the MTD for beagles and had to be lowered to 240 mg/kg on day 6. The high dose was associated with reduced food consumption, body weight loss, and clinical signs such as vomiting, salivation, pale gums, subdued behavior, and tremors. A few dogs in the high dose group were sacrificed early due to poor condition and/or weight loss. Several high dose dogs had cartilage lesions at one or more joints of the sort typically associated with fluoroquinolones. Most of the dogs in the high dose group had serum chemistry changes associated with liver damage and microscopic examination of the liver showed cholangitis/pericholangitis usually associated with minimal hepatocellular degeneration and single cell necrosis.

When gemifloxacin was administered orally to dogs for 13 weeks at doses of 60, 120, and 200 mg/kg, the target organ of toxicity was the liver. Decreased food consumption and reduced body weight gain or body weight loss were observed in drug treated dogs from the 120 and 200 mg/kg dose groups with a marginal effect in the low dose females. Vomiting was occasionally observed in some animals from all gemifloxacin groups and the incidence was dose-related. Salivation was observed in the mid and high dose dogs, particularly during the latter half of the study. The investigators believed that the salivating dogs may have been anticipating the vomiting, as salivation tended to begin just before gemifloxacin was administered or during dosing. Changes in serum chemistry parameters (especially ALT, but also AST, ALP, and GGT) indicative of hepatotoxicity were observed in drug-treated dogs, in some cases as early as week 7 of treatment. Microscopic changes in the liver included cholangitis/pericholangitis and hepatocellular degeneration/single cell necrosis, occurring most frequently in the portal areas. The NOEL for liver changes was not identified in this study. The liver changes appeared to be at least partially reversible, as signs of healing were observed in the 120 and 200 mg/kg dogs following a 4 week treatment free period and the livers of the 60 mg/kg dogs resembled the livers of control dogs after 4 weeks of no drug treatment. The sponsor has speculated that drug related material which precipitates out of the bile in dogs is responsible for the hepatic lesions. In another study submitted by the sponsor, Raman microspectroscopy was used to confirm that the precipitate observed in canine bile was related to gemifloxacin. Minimal to slight fatty atrophy of the bone marrow observed in some dogs from all treatment groups was attributed to stress, as was a modest reduction of total white blood cell count in the drug-treated females. Regardless of their origins, these effects were not observed in any gemifloxacin-treated dogs at the end of a 4 week drug-free period. The highest dose used in this study, 200 mg/kg, was associated with mortality (2 dogs were sacrificed early due to poor condition).

A second 13 week oral dog study was conducted to determine a NOAEL for gemifloxacin in canines for this treatment duration. The highest dose used in this study, 120 mg/kg, was associated with vomiting, reduced food consumption and body weight gain and mortality. It was reduced to 60 mg/kg during week 5 of dosing. Vomiting was seen at a lower incidence with less frequency at 30 mg/kg. Serum clinical chemistry measurements indicative of liver function/toxicity were increased in several dogs from the 120 mg/kg group and in one dog at 30 mg/kg as early as week 1 of treatment. Although the value for many of these parameters fell in the surviving high dose dogs after the dose was lowered to 60 mg/kg, several of the measurements were still higher

than baseline and control at the end of the study. Microscopic evaluation of the liver revealed minimal to slight single cell vacuolar degeneration in all animals from the 120/60 mg/kg group and in 1 male and 1 female from the 30 mg/kg group. Minimal to slight cholangitis/pericholangitis was observed in all of the 120/60 mg/kg males and 1 male at 30 mg/kg. Moderate cholangitis/pericholangitis with amorphous biliary deposits was seen in 1 female from the high dose group that survived for the entire dosing period. Histopathology data from a high dose female that was sacrificed early due to its poor condition revealed severe vacuolation of hepatocytes, amorphous biliary deposits, and biliary proliferation. The NOAEL in dogs for gemifloxacin administered orally each day for 13 weeks was 5 mg/kg.

When gemifloxacin was administered orally to dogs each day for 26 weeks, it was not associated with clinical signs of toxicity other than occasional vomiting at 10 and 60 mg/kg. Microscopic evaluation of the livers revealed minimal single cell vacuolar degeneration in 2/3 males and 3/4 females from the 60 mg/kg group. Mild cholangitis/pericholangitis was observed in 1/3 males and 1/4 females at 60 mg/kg. Increases in serum ALT were associated with these liver changes. The NOAEL for gemifloxacin was 10 mg/kg in this 26 week oral dog toxicity study.

Many of the clinical signs observed in dogs after IV administration of gemifloxacin daily for 4 days at doses of 10 and 30 mg/kg appeared related to histamine release, as has been observed in this species after IV administration of other quinolones. Tremor, redness of ears, lip licking, and head shaking were observed in dogs from the 10 and 30 mg/kg dose groups. Vomiting, facial swelling, redness of body, body extremities hot to touch, and subdued behavior were observed only at 30 mg/kg. The liver findings (pericholangitis, hepatocellular degeneration and single cell necrosis) with commensurate changes in serum clinical chemistry have been observed in dogs in other studies with gemifloxacin. Raman microspectroscopy indicated that brown material deposited in the bile duct of the high dose female dog appeared to be gemifloxacin and/or a metabolite. The NOAEL in this study was 3 mg/kg based on the clinical signs in the higher dose dogs and thymus atrophy in the higher dose females.

In a 14 day IV gemifloxacin toxicity study in dogs, the incidence and severity of histamine-related clinical signs (red ears, face, and body; swelling of ears, feet, and face; warm/hot ears and extremities, slight tremor, head shaking, lip licking, and agitation) was dose-dependant. The intensity of these effects at 30 mg/kg resulted in a reduction to 20 mg/kg on day 3 of administration. The clinical signs were much less severe and observed less frequently in the 10 mg/kg dose group. At 3 mg/kg, clinical signs (considered minimal in severity) were only observed on day 2 of administration in 2/3 dogs and included red, swollen, warm ears, and lip licking. Microscopic findings in the liver of a dog from the 20 mg/kg group included minimal focal pericholangitis and minimal proliferation of bile duct elements. This animal exhibited an increase in serum ALT (about 3 times baseline). A second dog in this high dose group also showed minimal proliferation of bile duct elements. The NOAEL for gemifloxacin was 10 mg/kg in this study, based upon histopathology.

Histamine-related clinical signs (swelling of forelimbs, ears, and face and redness of ocular membranes) were again observed during a 28 day IV gemifloxacin study in 2/6

dogs (both female) after 20 mg/kg was infused. Increases in plasma liver enzymes and microscopic changes in the liver (hepatocyte degeneration, minimal to slight pericholangitis, bile duct proliferation, presence of brown pigment) were observed in several dogs from the 10 and 20 mg/kg dose groups. The NOAEL for gemifloxacin given intravenously over one hour each day for 28 days to the dog was 2 mg/kg.

The sponsor conducted several experiments to investigate the precipitation of gemifloxacin in the canine biliary tract which appears to be responsible for the hepatic changes observed in dogs. It is likely that when crystals are formed in the bile ducts, the flow of bile is blocked; it can then leak out and damage nearby hepatocytes. When 30 mg/kg of gemifloxacin was administered IV to 3 dogs for 5 days, the amount of crystal deposition observed in each dog varied. Mild crystal formation in bile duct canaliculi in the periportal regions was observed in all of the dogs and these were often associated with brown pigment (believed to be evidence of intrahepatic cholestasis). The amount of crystal formation in the bile ducts was mild to moderate in one dog, moderate in a second dog, and minimal (seen only in one bile duct) in the third. The presence and severity of cholangitis/pericholangitis and hepatocellular degeneration/necrosis correlated directly with the amount of bile duct crystal formation and they were observed mainly in periportal areas. Crystals were occasionally associated with hepatocytes or were seen within hepatocytes. In another investigation, liver sections from a dog in the 30 mg/kg gemifloxacin group in the 4 day IV study discussed previously were examined by scanning electron microscopy and an X-ray energy spectrometer. Magnesium and fluorine were associated with the crystals, but not with areas of the same liver specimen where no crystals were present. Gemifloxacin contains fluorine and fluoroquinolones are known to chelate magnesium. This observation suggested that the crystals in the bile duct of the gemifloxacin-treated dog were gemifloxacin (and/or a metabolite) in a complex with magnesium. Finally, gemifloxacin is more soluble in dog bile from the gallbladder than hepatic dog bile, with the pH difference a factor in the differential solubility. The mean pH (at room temperature) of canine gallbladder bile was 7.12 ± 0.09 and of hepatic bile was 8.44 ± 0.12. The mean solubility of gemifloxacin free base in gallbladder bile was 1.70 ± 0.06 mg/ml and in hepatic bile it was 0.60 ± 0.13 mg/ml, suggesting that gemifloxacin crystals are more likely to form in the liver than in the gallbladder. This is consistent with observations from gemifloxacin toxicity studies in dogs.

The reader may be aware that microscopic changes were also reported in the livers of dogs that received the fluoroquinolone trovafloxacin. Use of this drug in humans has been restricted due to rare, but serious human hepatotoxicity. The pattern of hepatotoxicity observed in dogs (and occasionally rats) that were exposed to gemifloxacin is different from that which was observed following trovafloxacin treatment of dogs. None of the reports of the trovafloxacin studies in dogs that had microscopic changes in their livers mentioned that crystals were observed in the biliary tract. Also, the histopathologic lesions in the livers of dogs given trovafloxacin tended to be centrilobular (more associated with the central vein) while those reported for gemifloxacin have been periportal (more associated with the bile duct). Additionally, hepatic lesions in dogs were generally not observed without long term exposure to

trovafloxacin. Microscopic liver changes were reported following shorter periods of gemifloxacin administration and the incidence was also higher.

Gemifloxacin has been demonstrated to prolong the QTc interval in beagle dogs. A 30 mg/kg IV dose given over 30 minutes was associated with a transient increase in blood pressure and heart rate followed by a decrease in blood pressure with heart rate falling to baseline despite the infusion continuing. A 16% increase in the QTc interval was observed 5 minutes after the end of infusion and the QRS complex was increased about 20 minutes after the end of infusion. A separate pharmacokinetic study in male beagle dogs receiving 30 mg/kg of gemifloxacin over 30 minutes demonstrated an average plasma concentration of 6.2 g/ml (around 4 times the average human Cmax following multiple oral gemifloxacin doses of 320 mg, or 2.4 times the upper end of the range for human Cmax) and an average concentration in heart tissue of 51 g/g. The no-effect dose for cardiovascular changes in the beagle dog was 10 mg/kg, when gemifloxacin was given as an IV infusion over 30 minutes. Following a single oral dose of 50, 100, or 200 mg/kg of gemifloxacin to beagles, the QRS complex duration increased in a manner that was not dose-dependant (6, 15, and 10 msec, respectively). These increases were observed beginning at about 1.3 hours and lasting until 4.7 hours after administration of the low gemifloxacin dose and starting 2-3 hours after the higher doses were given and lasting throughout the monitoring period. No increases in QTc were observed following these oral doses of gemifloxacin. The Cmax after these doses ranged from 5.04-6.76 g/ml, with Tmax of 3-5 hours. Following doses of $\leq$40 mg/kg (Cmax$\leq$3.78 g/ml), no changes in either QRS or QTc were observed. In a study of isolated canine Purkinje fibers, gemifloxacin increased the action potential duration by about 10% at 100 M, but no change occurred at 10 M. Grepafloxacin and sparfloxacin (at both 10 and 100 M) were associated with greater increases in APD (approximately 30% and 60%, respectively, at APD90). Unlike trovafloxacin and grepafloxacin, gemifloxacin was not associated with a decrease in maximum upstroke velocity.

Male fertility in rats was not affected by oral doses of gemifloxacin up to 270 mg/kg (about 3-fold higher than average human exposure based on AUC after multiple 320 mg doses) given for at least 63 days prior to mating. This was the highest dose tested in males due to the dose-limiting toxicity of crystal nephropathy. Female rat fertility was not affected at oral doses up to 750 mg/kg (about 4 times the average human exposure based on AUC), given for at least 14 days prior to mating until day 17 of pregnancy. Doses $\geq$270 mg/kg appeared slightly toxic to sires and dams ("noisy breathing" noted in some rats) and there was an increased pre-coital interval in the mid and high dose groups. Gemifloxacin was not associated with increased pre- or post-implantation loss and did not induce fetal malformations in the offspring of dams given up to 750 mg/kg. However, the drug was fetotoxic at 750 mg/kg (reduced average fetal body weights and increased incidences of incomplete skeletal ossification, consistent with fetal growth retardation). Doses of gemifloxacin up to 270 mg/kg in dams did not appear to be fetotoxic in this study.

In mice, no adverse effects on dams or fetuses were observed at gemifloxacin doses up to 250 mg/kg when the drug was administered orally from days 6-15 of gestation. A gemifloxacin dose of 450 mg/kg (about 2 times the average human exposure

based on AUC) was associated with maternal mortality (but not clinical signs of toxicity including reduced body weight gain or food consumption) and fetotoxicity. Mean fetal body weights at 450 mg/kg were significantly reduced compared to control and delays in skeletal observation were observed, indicative of fetal growth retardation.

When gemifloxacin was administered IV to rabbits during days 7-19 of gestation, reduced mean food consumption in the does from the 40 mg/kg group (the highest dose tested, about 3 times the average human exposure based on AUC) appeared to be a sign of slight maternal toxicity. The mean weight of fetuses from this dose group was less than controls. This could be related to the reduction in maternal food consumption or a sign of slight fetotoxicity. Gemifloxacin was not associated with an increase in external, visceral, or skeletal malformations in rabbit fetuses from does given up to 40 mg/kg/day via intravenous infusion.

The potential effects of gemifloxacin on organogenesis and perinatal and postnatal development were evaluated in rats. Dams were dosed orally from day 6 of gestation through day 20 of lactation. In the 750 mg/kg gemifloxacin dose group (the highest dose tested), F0 dams consumed significantly less food than controls and gained less weight during gestation. Despite continued gemifloxacin treatment, these rats consumed a similar amount of food as controls during the lactation period and mean body weight did not differ significantly between the 2 groups by day 8 of lactation. Pup birth weights in the 750 mg/kg group were significantly lower than control, but by day 21 of lactation, body weights no longer differed significantly and skeletal ossification did not differ between F1 rats in the control and high dose groups. These observations suggest that the fetal growth retardation observed in this study and in the previous rat fertility and embryonic development study is reversible. Eye malformations and dome-shaped head were observed in some pups from the 750 mg/kg group in the current study (overall, 14 pups from 5 litters). These malformations were not seen in the previous rat fertility and embryonic development study, but it should be noted that the serum concentrations in the current study are higher than the plasma concentrations seen in the former study (exposure was about 8 times greater than the average human exposure based on AUC). The reason for the difference is not clear- the same strain of rat was used for both studies although they were from different suppliers. The only other difference was that a larger dose volume was used in the current study. Attainment of developmental landmarks and behavioral test results (startle response, passive avoidance learning/retention, spontaneous activity) in the F1 pups were not affected by maternal gemifloxacin treatment from day 6 of pregnancy throughout the lactation period at doses up to 750 mg/kg. Reproductive function in the F1 generation was also unaffected at the same doses. Pharmacokinetic data from pregnant and lactating rats indicate that gemifloxacin crosses the placenta and is excreted in the milk of this species.

Gemifloxacin did not induce reverse mutation at the *his* locus of the *Salmonella* strains tested (TA98, TA100, TA1535, TA1537). However, due to the potent cytotoxicity of gemifloxacin, the concentrations used in this study were so low ($\leq$ 20 ng/plate) that the results have little meaning. It should also be noted that other fluoroquinolones have had negative test results in these bacterial strains, but have tested positive in *Salmonella* strain TA102. Unscheduled DNA synthesis was not observed in

hepatocytes from rats treated with single oral 1000 or 2000 mg/kg doses of gemifloxacin when cells were harvested 2-4 or 12-14 hours after dosing. Like some other fluoroquinolones, gemifloxacin induced mutation at the TK locus of L5178Y mouse lymphoma cells in the presence or absence of metabolic activation. Incubation with gemifloxacin *in vitro* also caused statistically significant increases in the number of cultured human peripheral blood lymphocytes with chromosome aberrations regardless of metabolic activation. Gemifloxacin did not induce micronucleus formation in polychromatic erythrocytes (PCEs) from the bone marrow of mice given 2 consecutive daily doses of up to 50 mg/kg via intraperitoneal injection. However, micronucleus formation in the bone marrow of rats was observed following oral administration of the drug for 2 consecutive days at doses of $\geq$800 mg/kg/day. The no effect level for micronucleus formation was 400 mg/kg/day. Gemifloxacin also induced micronucleus formation in rat bone marrow PCEs following 2 days of intravenous dosing at 40 and 80 mg/kg/day. The no-effect gemifloxacin dose was 20 mg/kg/day in this study. The doses of gemifloxacin associated with micronucleus formation in both the oral and IV rat studies were toxic to the rats' bone marrow. The percentage of PCEs (out of the total number of erythrocytes) in the bone marrow was clearly reduced at oral doses $\geq$800 mg/kg/day, as was the reticulocyte count in peripheral blood. Bone marrow toxicity (reduction of the % PCE out of total erythrocytes) was also observed at the IV doses of 40 and 80 mg/kg/day. The sponsor has suggested that reticulocyte count might be a useful surrogate for gemifloxacin-induced bone marrow toxicity in situations (i.e., a human clinical trial) where a less invasive method to monitor bone marrow toxicity would be desirable. They are apparently assuming that micronucleus formation does not occur at doses of gemifloxacin that are not toxic to the bone marrow; thus if no evidence of bone marrow toxicity (reduction of reticulocyte count) is observed in humans, micronucleus formation would be unlikely to occur at doses of gemifloxacin used clinically. The sponsor has cautioned against using either Cmax or AUC in isolation to try to predict *in vivo* micronucleus formation. In the IV rat micronucleus study, the no-effect dose for gemifloxacin was 20 mg/kg which gave a Cmax of 31.1 $\pm$ 3.72 g/ml and an AUC of 13.8 $\pm$ 1.35 gh/ml. This Cmax is about 3 times higher than that observed for the lowest dose that induced micronuclei in the oral study (800 mg/kg), although the AUC after the oral 800 mg/kg dose was over twice as high as that observed following the 20 mg/kg IV dose. The lowest gemifloxacin dose in the IV rat study that induced micronuclei was 40 mg/kg, giving a Cmax of 63.6 $\pm$ 20.1 g/ml and an AUC of 35.2 $\pm$ 3.23 gh/ml. This AUC is comparable to that observed at the no effect dose in the oral rat micronucleus study (400 mg/kg), but the Cmax after the 40 mg/kg IV dose was over 5 times higher than that observed after the 400 mg/kg oral dose. The Cmax after the 800 mg/kg oral dose in rats was about 6 times the average human Cmax, and the rat AUC was about 9.5 times higher than human. After the 40 mg/kg IV dose in rats, Cmax and AUC were about 42 and 4 times greater than the respective average human pharmacokinetic parameters.

Although the members of the fluoroquinolone class have generally been

positive in one or more of the *in vitro* assays for genotoxicity (particularly those used to identify clastogens), most of those tested *in vivo* in the mouse micronucleus assay did not induce micronuclei in the target cells. This includes the fluoroquinolones approved as human drugs in the U.S. Thus, some members of the review team expressed concern regarding the positive rat micronucleus data generated with gemifloxacin. The gemifloxacin data was submitted to the CDER Genetic Toxicology Committee for their review. They agreed that the compound is a genetic toxicant and did not believe that additional studies that would only serve to confirm this were necessary. The committee also felt that it would be useful to compare the gemifloxacin genotoxicity data with that from other fluoroquinolones. It should be noted that none of the other approved fluoroquinolones were tested in a rat micronucleus assay (only mice were used if the compound was tested at all in this type of experiment). Additionally, there are frequently no toxicokinetic data demonstrating the exposure of the mice to the older drugs under the dosing conditions of the micronucleus tests. Thus, it is not possible to draw comparisons between exposure to these marketed drugs vs. exposure to gemifloxacin (e.g., in some assays where large oral doses were used, much of the test compound may not have been absorbed by the mice). One should also recall that as a class, the fluoroquinolones are considered clastogenic and the rodent micronucleus assay is a means of identifying clastogenic compounds, so any of these drugs could theoretically be positive in the assay if adequate exposure to the target occurs. The *in vitro* assays conducted prior to the rat micronucleus studies had already indicated that, like other quinolones, gemifloxacin is a clastogen. Some of the fluoroquinolones previously approved as human drugs have been tested in rodent bioassays for carcinogenicity, including ciprofloxacin, levofloxacin, norfloxacin, sparfloxacin, and gatifloxacin. None of these was a rodent carcinogen in spite of their genotoxicity. The positive rat micronucleus data with gemifloxacin may also be considered in light of several approved human drug products which have not only been positive or have demonstrated equivocal findings in one or more genotoxicity assays, but are rodent carcinogens. These include the substituted benzimidazole proton pump inhibitors lansoprazole (Prevacid), omeprazole (Prilosec), and rabeprazole (Aciphex), as well as the antihistamine loratadine (Claritin). The pharmacology reviewer believes it unlikely that gemifloxacin is a greater genotoxic hazard to humans than any of the other fluoroquinolones when these drugs are used as recommended (e.g., short term antimicrobial therapy) based on a positive result in a single type of clastogenicity assay. Consequently, the pharmacologist does not recommend that the Division consider the results of the rat micronucleus assay to be an issue for approving this product. A phase 4 commitment to clarify the relationship of gemifloxacin to the other fluoroquinolones should be acceptable to address the concerns of the review team. In order to make a fair comparison between gemifloxacin and other marketed fluoroquinolones, the Division has recommended that the sponsor conduct a rodent micronucleus assay with

several of these compounds and collect toxicokinetic data. The clastogenic potential of gemifloxacin and other fluoroquinolones at various levels of exposure can be compared directly in this study.

Gemifloxacin was less potent than other quinolones such as ciprofloxacin, enoxacin and lomefloxacin at inducing phototoxic reactions in hairless mice when animals receiving single oral doses of these drugs were exposed to UVA radiation. Ciprofloxacin appeared only slightly more phototoxic than gemifloxacin and both enoxacin and lomefloxacin were more phototoxic than either gemifloxacin or ciprofloxacin. A 100 mg/kg single dose of gemifloxacin did not appear to be phototoxic to the hairless mice, but a 200 mg/kg single dose of gemifloxacin was associated phototoxic reactions in hairless mice when combined with UVA exposure. As gemifloxacin also has some absorption in the UVB range, the sponsor was advised to perform an additional phototoxicity study using simulated sunlight which would cover all relevant portions of the UV spectrum. A study using simulated sunlight was conducted so that hairless mice treated with gemifloxacin and radiation would be exposed to both UVA and UVB. Under the conditions of this study, gemifloxacin was not associated with phototoxicity in hairless mice at doses up to 100 mg/kg (13 weeks of drug + UV). A higher dose (200 mg/kg) of gemifloxacin was not tolerated due to obstructive tubular nephropathy. This nephropathy was also observed in one male mouse from the 100 mg/kg gemifloxacin group. Gemifloxacin was not photocarcinogenic in hairless mice. At doses of up to 100 mg/kg with concurrent exposure to simulated sunlight, gemifloxacin did not shorten the time to observation of UV-induced tumors in hairless mice or increase their number or severity. The positive control substance, lomefloxacin, clearly reduced the time until observation of UV-induced tumors and increased tumor yield in the mice. The concentration of gemifloxacin in the skin of the mice at Cmax, around the time of irradiation (on the days when drug was given prior to UV exposure), following a 100 mg/kg dose was about 6.06 g/g when the skin data from a supportive toxicokinetic study were averaged. Plasma levels following a 100 mg/kg dose were about 1.13 g/ml in the mice around the time of irradiation. There are no data on gemifloxacin skin levels in humans, but the mouse plasma gemifloxacin levels are in the expected range of human plasma Cmax levels following multiple 320 mg oral doses.

In a test of active systemic anaphylaxis, guinea pigs were sensitized to gemifloxacin when it was administered subcutaneously with Freund's complete adjuvant (regardless of whether it was conjugated to human serum albumin). When gemifloxacin was administered orally, however, sensitization of the guinea pigs did not occur. Rats receiving serum from mice sensitized to gemifloxacin alone did not demonstrate passive cutaneous anaphylaxis reactions if the mice were sensitized via either oral administration of gemifloxacin or IP administration in combination with $Al(OH)_3$). Gemifloxacin conjugated to human serum albumin was antigenic in this model (in 3/5 animals) if gemifloxacin conjugated to rat serum albumin was used for the challenge- neither gemifloxacin alone or rat serum albumin alone elicited a response when used as a challenge antigen. Serum from mice sensitized with a conjugate of gemifloxacin and human serum albumin agglutinated sheep red blood cells coated with a gemifloxacin/rat

serum albumin conjugate, but not if the cells were coated with rat serum albumin alone. This suggests that gemifloxacin may have the potential to induce sensitization, though it appears unlikely that it would occur when the drug is administered orally. Parenteral routes of gemifloxacin administration appear more antigenic and conjugating the drug to protein increased the antigenic potential.

A number of the toxic effects observed in animals following gemifloxacin administration are similar to those that have been seen with other quinolones. These include the induction of arthropathy in juvenile dogs, convulsions and other CNS disturbances, fetal growth retardation in mice and rabbits, and malformations and fetotoxicity in the offspring of female rats dosed with the drug. Gemifloxacin caused periportal liver injury in dogs and rats that was associated with precipitation of drug-related substance in the bile ducts. The frequency and incidence of this effect was much greater in the dog studies than in rats. In dogs, hepatotoxicity has been observed at exposure levels that are achieved in humans following multiple 320 mg doses of gemifloxacin. The sponsor believes that precipitation of gemifloxacin in human bile is not likely to occur, but the Medical Officers in the Division are evaluating this possibility (and pondering label recommendations for clinical use of this product), particularly because liver enzyme elevations have been seen in some human subjects that received doses of gemifloxacin greater than 320 mg during clinical trials. The dose-limiting toxicity of gemifloxacin in rats was crystal nephropathy, which has been observed with several other fluoroquinolones. It can occur in rats at gemifloxacin exposure levels that are achieved in humans. As with the other members of the quinolone drug class, crystal nephropathy is much less likely to occur in humans than rats due to the more acidic pH of human urine compared to these rodents. The labels for fluoroquinolone drug products generally contain a recommendation that patients who take these drugs should stay well hydrated to prevent highly concentrated urine from developing and this recommendation is already present in the gemifloxacin label. The potential for gemifloxacin to prolong the QTc interval and the duration of the QRS complex was demonstrated in dogs. QTc prolongation has also been observed in some clinical studies with gemifloxacin. In dogs, the approved drugs sparfloxacin and moxifloxacin appeared to be more potent prolongers of the QTc interval than gemifloxacin.

RECOMMENDATIONS: The nonclinical data provide no grounds for denying the approval of gemifloxacin, so the pharmacologist does not recommend that course of action. The nonclinical data for gemifloxacin are generally similar to other quinolones marketed for clinical use. The label contains appropriate cautions regarding potential quinolone-related toxicities such as CNS effects, tendon rupture, and juvenile arthropathy. Additionally, the label will contain cautionary language against exceeding the recommended dose due to the observation of liver enzyme elevations in test subjects that have received higher doses of gemifloxacin. Specific labeling recommendations from the pharmacologist are presented in detail above. So that the *in vivo* clastogenic potentials of marketed

fluoroquinolones can be compared to gemifloxacin in a straightforward manner, the sponsor has been requested to conduct a rodent micronucleus study (including toxicokinetic evaluations) using several of these drugs as a Phase 4 commitment.

Amy L. Ellis, Ph.D.
Pharmacologist, HFD-520

Orig. NDA
cc:
HFD-520
HFD-590
HFD-104
HFD-340
HFD-520/Pharm Team Ldr/Osterberg
HFD-590/Pharm Team Ldr/Hastings
HFD-520/Pharm/Ellis
HFD-590/Pharm/Hundley
HFD-590/MO/Powers
HFD-520/Chem/Sloan
HFD-590/CSO/Kimzey
HFD-590/Micro/Dionne

Concurrence Only:
HFD-520/REOsterberg
HFD-520/LGavrilovich

Histopathology Inventory for NDA #21,158

Study	RSD-100V33/2	RSD-100XTB/2	RSD-100Z4L/1	RSD-100XOR/2	RSD-100TT5/1	RSD-100ZRV/2
Species	Rat	Rat	Dog	Dog	Dog	Dog
Adrenals	X*	X*	X	X*	X*	X*
Aorta	X	X	X	X	X	X
Bone Marrow smear			X	X		X
Bone (femur)		X	X	X	X	X
Brain	X*	X*	X	X*	X*	X*
Cecum	X	X	X	X	X	X
Cervix			X			X
Colon	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X
Epididymis	X*	X*	X	X	X*	X*
Esophagus	X	X	X	X	X	X
Eye	X	X	X	X	X	X
Fallopian tube						
Gall bladder			X	X	X	X
Gross lesions	X	X	X	X		X
Harderian gland						
Heart	X*	X*	X	X*	X*	X*
Ileum	X	X	X	X	X	X
Injection site	X	X			X	X
Jejunum	X	X	X	X	X	X
Kidneys	X*	X*	X	X*	X*	X*
Lachrymal gland			X	X		
Larynx			X	X	X	X
Liver	X*	X*	X*	X*	X*	X*
Lungs	X	X	X	X	X	X
Lymph nodes, cervical	X				X	
Lymph nodes mandibular		X	X	X		X
Lymph nodes, mesenteric	X	X	X	X	X	X
Mammary Gland	X	X	X	X	X	X
Nasal cavity						
Optic nerves	X	X	X		X	X
Ovaries		X*	X	X*		X*
Pancreas	X	X	X	X	X	X
Parathyroid	X	X	X	X	X	X
Peripheral nerve						
Pharynx						
Pituitary	X	X	X	X	X	X
Prostate	X*	X*	X	X*	X*	X*
Rectum			X	X	X	X
Salivary gland	X	X	X	X	X	X
Sciatic nerve	X	X	X	X	X	X
Seminal vesicles	X*	X*				
Skeletal muscle	X	X	X	X	X	X
Skin	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X
Spleen	X	X*	X	X	X	X
Sternum	X	X	X	X	X	X
Stomach	X	X	X	X	X	X
Testes	X*	X*	X	X*	X*	X*
Thymus	X*	X*	X	X	X*	X*
Thyroid	X	X	X	X*	X	X
Tongue			X	X	X	X
Trachea	X	X	X	X	X	X
Urinary bladder	X	X	X	X	X	X
Uterus		X	X	X*		X
Vagina		X	X	X		X
Zymbal gland						

NDA 21,158-000/Factive (gemifloxacin)

* organ weight obtained